Remarks

The Final Office Action mailed April 9, 2002 has been received and reviewed. Claims 1 through 19 are pending in the application. Claims 4 through 10, 12, 14 through 15, and 18 through 19 have been withdrawn from consideration. Claims 1 through 3, 11, 13, 16, and 17 stand rejected. Reconsideration is respectfully requested.

Claims 1-3 and 13 were rejected under 35 U.S.C. § 102(b) as being anticipated by Bilej et al. (Bilej et al., Identification of TNF-Like Activity in Earthworms, European Cytokine Network, March-April 1994, vol. 5 no. 2, page 99). Claims 11 and 16-17 were rejected under 35 U.S.C. § 102(b) as being anticipated by Bilej et al. (Bilej et al., Identification of a cytolytic protein in the coelomic fluid of Eisenia foetida earthworms, Immunology Letters, Vol. 45 (1995), p. 128). Applicants respectfully traverse the rejections as hereinafter set forth.

Claim 1 is directed to "[a] peptide comprising at least 9 contiguous amino acids of SEQ. ID. NO. 1." The Final Office Action indicates "it would be inherent that the CCF-1 protein as taught by Bilej et al. would comprise at least 9 contiguous amino acids of SEQ ID No: 1" and "the burden is on the applicant to show a novel or unobvious difference between the claimed product and the product of the prior art." (Final Office Action, mailed April 9, 2002, pages 3 and 4). Bilej et al. discloses a semi-pure active fraction obtained from coelomic fluid, while claim 1 is limited to the peptide of SEQ ID NO: 1 which is not the same as the coelomic fluid fraction disclosed in Bilej et al. Thus, the claimed peptide is different from the coelomic fluid fraction disclosed in the cited prior art.

Attached to this amendment is Exhibit A indicating that the claimed peptide (rCCF-1) does not possess significant cytolytic (i.e., hemolytic) activity (*See*, Fig. 2, Exh. A) while the Bilej et al. reference indicates that the "[c]oelemic fluid of the earthworm *Eisenia foetida* (*Annelida*) contains strong proteolytic, hemolytic, bacteriolytic and cytolytic factors." (Bilej et al.) Accordingly, since the claimed peptide does not possess the same functional characteristics as the coelomic fluid disclosed in Bilej et al., applicants request reconsideration and withdrawal of the anticipation rejection of claim 1.

Since claims 2 and 3 depend from claim 1, claims 2 and 3 are also not anticipated by Bilej et al. and applicants request reconsideration and withdrawal of the anticipation rejections of claims 2 and 3.

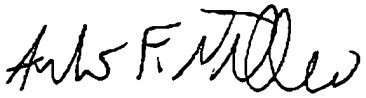
Claim 11 claims a pharmaceutical composition including peptides selected from the group of peptides of SEQ ID NO: 1, SEQ ID NO: 3 or a fragment or epitope thereof. Attached to this amendment is Exhibit A indicating that the claimed peptide domains do not possess significant cytolytic (i.e. hemolytic) activity (*See*, Exhibit A), while the cited prior art reference indicates that the disclosed coelomic proteins do possess cytolytic and hemolytic activities. (*See*, Bilej et al.) Therefore, since the applicants have established the novelty of the claimed invention over the cited prior art reference, the claimed pharamaceutical composition cannot be anticipated, and applicants request reconsideration and withdrawal of the anticipation rejection of claim 11.

Since claims 13, 16 and 17 depend from novel independent claim 11, claims 13, 16 and 17 are also not anticipated by the cited prior art reference and applicants respectfully request reconsideration and withdrawal of the anticipation rejections of these claims.

Conclusion

If any questions remain after consideration of the foregoing, the Office is kindly requested to contact applicants' attorney at the address or telephone number given herein.

Respectfully submitted,



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Date: July 19, 2002
Attachment: Exhibit A

EXHIBIT A

Coelomic fluid of annelids exerts a large variety of biological functions including proteolytic, antibacterial, hemolytic, and cytolytic properties. Recently three hemolytic proteins of *Eisenia fetida* coelomic fluid (ECF) H_1 (46 kDa), H_2 (43 kDa), and H_3 (40 kDa) sharing lectin-like activities have been identified (Eue *et al.* 1998). While all three molecules display heat labile hemolytic activity, H_3 hemolysin shows also heat stable agglutinin activity.

Independently, a 42-kDa cytolytic molecule named CCF-1 for coelomic cytolytic factor acting as a pattern recognition molecule for Gram negative and Gram positive bacteria as well as yeast has been cloned. Accordingly, by binding LPS, muramyl residues of peptidoglycan, b 1,3-glucans, and N'N-diacetylchitobiose, CCF-1 triggers the activation of the prophenoloxidase Invertebrate defense mechanisim (Bilej *et al.* 2001; Beschin *et al.* 1998). Since hemolytic and cytolytic proteins are often functionally related in annelids (Kauschke and Mohrig 1987) our investigation focused on the possible role of CCF-1 in hemolysis as well as the interaction of CCF-1 with H_1, H_2, and H_3.

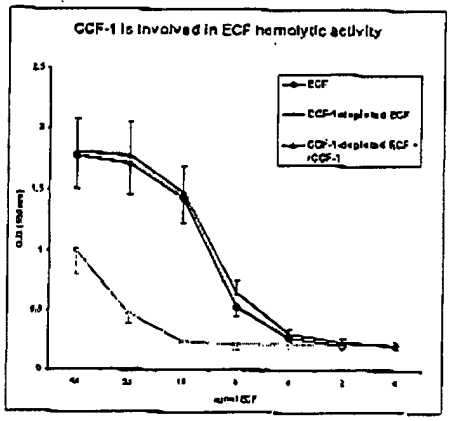


Fig. 1

Since CCF-1 has strong glucan-binding properties ECF was preincubated with insoluble curdlan (β-1,3-glucan) to remove CCF-1. The hemolytic activity of CCF-1-depleted ECF upon rat erythrocytes is considerably decreased but can be completely recovered by addition of rCCF-1.

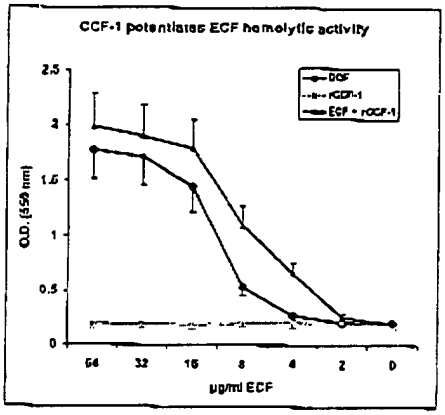


Fig. 2

Though rCCF-1 does not exert any hemolytic activity on murine, rat, guinea pig, rabbit, and sheep erythrocytes it potentiates hemolytic activity of ECF (results shown for rat erythrocytes, rCCF-1 was given at 1 mg/ml).

Fig. 3A

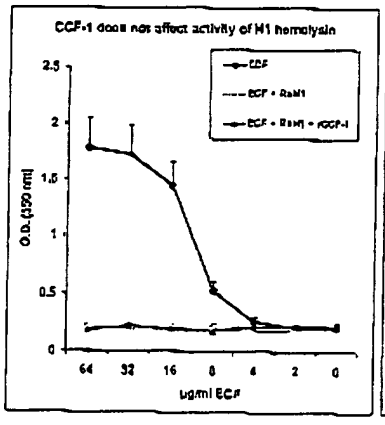


Fig. 3B

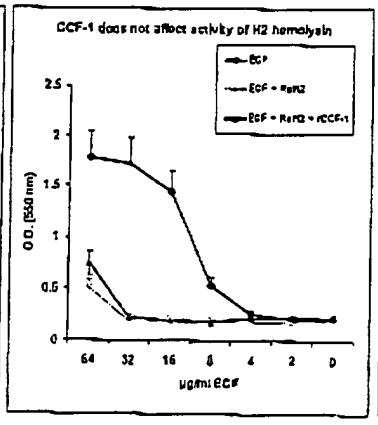
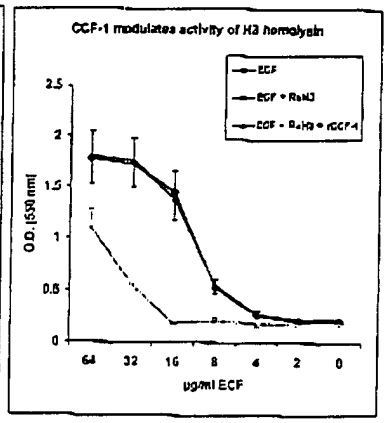


Fig. 3C



ECF hemolytic activity is inhibited by antibodies raised against three hemolytic CF proteins (H_1, H_2, H_3). Recombinant CCF-1 does not affect the neutralization of the hemolytic properties of H_1 and H_2 by rabbit anti-H_1 and anti-H_2 antibodies (RaH_1, RaH_2; fig. 3A/B). In contrast rCCF-1 raises the activity of H_3 and neutralizes the inhibition caused by rabbit anti-H_3 antibody (RaH_3; rCCF-1 was given at 1 µg/ml; fig. 3C).

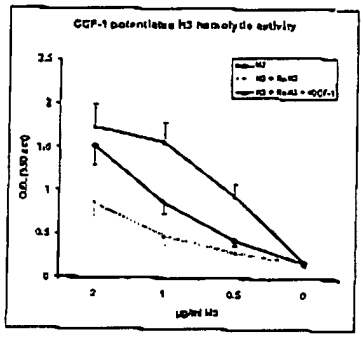


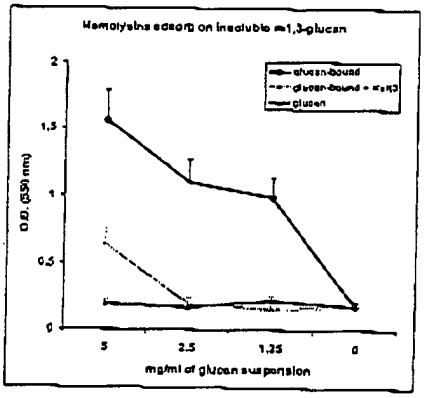
Fig. 4

To further evidence the effect of rCCF-1 on H3 hemolysin ECF proteins were electroseparated on native gel. The H3 hemolysin was electroeluted, dialyzed, and the hemolytic activity was assessed on rat erythrocytes. We showed that rCCF-1 can neutralize the inhibition caused by rabbit anti-H3 antibody (rCCF-1 was given at 1 mg/ml; fig.4).

Fig. 5

Monosaccharides	% inhibition
Glucose (Glc)	NI
Galactose (Gal)	NI
N-acetylglucosamine (GlcNAc)	6.1 (n.s.)
Fucose	NI
Rhamnose	10.1 (n.s.)
Mannose	NI
Mannitol	NI
Disaccharides	
Lactose (Gal-α-1,4-Glc)	NI
Sucrose (Glc-β-1,2-Fru)	NI
Chitobiose (GlcNAc-β-1,4-GlcNAc)	NI
Polysaccharides	
Laminarin (β-1,3-Glc link)	NI
Zymosan (β-1,3-Glc link)	26.5
Curdlan (β-1,3-Glc link)	96.5
Cellobiose (β-1,4-Glc link)	NI
Lichenan (β-1,4-Glc link)	NI
Chitin (β-1,4-GlcNAc link)	6.2 (n.s.)

Saccharides were given at 100 µg/ml, data indicate percentage of inhibition of ECF dilution exerting 50 % of hemolytic activity (NI – not inhibiting ; zymosan, curdlan, and chitin are insoluble ; n.s. – not significant).



CCF-1 displays lectin-like properties and binds efficiently some polysaccharides. Investigating the inhibition of the hemolysis by various saccharides and polysaccharides (given at 100 µg/ml) we found that insoluble but not soluble β-1,3-glucans significantly decreased the hemolytic activity of ECF. On the other hand the material adsorbed on the surface of insoluble β-1,3 glucan particles exerts hemolytic activity that is inhibited by RaH_3 antibody (fig. 5). This might reflect immobilization of CCF-1 and H_3 on insoluble glucan particles: at low concentration the contact between curdlan particles and erythrocytes is not sufficient enough while at higher concentration the possibility to interact with erythrocyte membranes becomes more frequent.

Conclusions:

- The CCF-1 protein is not hemolytic, but potentiates the hemolytic activity of *Eisenia fetida* coelomic fluid possibly by interacting directly with the H_3 hemolysin. This is reminiscent of the observation that rCCF-1 is not lytic but is required for ECF to exert lytic activity on L929 cells.
- The hemolytic activity of ECF is blocked by insoluble but not by soluble β-1,3-glucan, suggesting that this activity requires the recognition of saccharide structure on red blood cell surface with a high ligand density. Hemolytic activity of insoluble β-1,3-glucan-bound material might reflect the immobilization of H_3 hemolysin directly or *via* CCF-1 and equilibrium between erythrocytes and glucan particles.
- Whether the inhibition of hemolytic ECF activity by β-1,3-glucan reflects the recognition of this saccharide moiety on red bood cell surface, or the impairment of CCF-1 activity blocking the interaction of the latter with hemolysin is currently under investigation.
- We hypothesize that CCF-1, by binding to saccharide moieties on red blood cells, favors the interaction of hemolysins with erythrocytes membranes. Similarly, CCF-1 may bind saccharide moieties on extracellular matrix or on L929 membranes facilitating the access of ECF cytolytic proteins to L929 membrane molecules (sphingomyelin?)

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